Atty. Dkt. No 065691-0387

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Christophe de ROMEUF

Title: TREATMENT OF PATHOLOGIES WHICH ESCAPE THE IMMUNE RESPONSE, USING OPTIMIZED ANTIBODIES

Appl. No.: 10/527,666

Filing Date: 08/01/2005

Examiner: Crowder, Chun

Art Unit: 1644

TRANSMITTAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith are the English translation of the specification of French priority no. 03 07066 filed on June 12, 2003 and a Declaration verifying that the English translation filed with the USPTO is a true and correct translation of the priority document.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Respectfully submitted,

Date May 11, 2006

By _____

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5300
Facsimile: (202) 672-5399

Matthew E. Mulkeen
Attorney for Applicants
Registration No. 44,250

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial N° : 10/527,666

Filed : September 15, 2003

For : Treatment of pathologies which escape the immune response, using optimized antibodies

DECLARATION

I, Nicolas Torno, c/o Cabinet Regimbeau, 20 rue de Chazelles, 75017 Paris (France), hereby declare that I am well acquainted with the French and English languages and hereby certify that to the best of my knowledge and belief the following is a true translation of French priority n° 03 07066 filed on June 12, 2003.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date : March 17, 2006

Nicolas TORNO

The present invention relates to the use of optimized human or humanized chimeric monoclonal antibodies which are produced in selected cell lines, said antibodies having strong affinity for the CD16 receptor of the effector cells of the immune system, and also being able to induce the secretion of cytokines and of interleukins, in particular IFNγ or IL2, for the treatment of pathologies for which the target cells express only a low antigenic density and in which the effector cells can only be recruited in small amounts.

Immunotherapy by means of monoclonal antibodies is in the process of becoming one of the most important aspects of medicine. On the other hand, the results obtained during clinical trials appear to be contrasting. In fact, the monoclonal antibody may prove to be insufficiently effective. Many clinical trials are stopped for various reasons such as a lack of effectiveness, and side effects that are incompatible with use in clinical therapy. These two aspects are closely linked given that antibodies that are not very active are administered at high dose in order to compensate for this and to obtain a therapeutic response. The administration of high doses not only induces side effects, but it is not very economically viable.

These are major problems in the human or humanized chimeric monoclonal antibody industry.

Now, this problem is exacerbated for a certain number of pathologies for which the antigenic density expressed by the target cells is low and/or the low number of available and activated effector cells rendering technically impossible the use of antibodies

for therapeutic purposes with the antibodies currently available. For example, in Sézary syndrome, the specific antigen, KIR3DL2, is weakly expressed (only approximately 10 000 molecules). The expression of tumor antigens may also be negatively regulated, such as HER2-neu in breast cancer. Moreover, when it is sought to inhibit angiogenesis via the targeting of VEGFR2, few molecular targets are effectively accessible since the receptor is internalized. Similarly, tumor antigen-specific peptides presented by HLA class 1 or class 2 molecules, for example in the case of carcinomas, melanomas, ovarian cancers, prostate cancers, are generally expressed very little at the surface of the target tumor cells. Finally, another situation can occur in viral infections in which the cells infected with certain viruses (HBV, HCV, HIV) express only a few viral molecules on their membrane.

This problem also arises for all pathologies which exhibit a decrease in the number of NK cells, or in their activity or in their number of CD16s (Cavalcanti M et al., Irreversible cancer cell-induced functional anergy and apoptosis in resting and activated NK cells, Int J Oncol 1999 Feb; 14(2): 361-6). Mention may be made, for example, of chronic myeloid leukemias (Parrado A. et al., Natural killer cytotoxicity and lymphocyte subpopulations in patients with acute leukemia, Leuk Res 1994 Mar; 18(3): 191-7), pathologies associated with the environment that target in particular individuals exposed to polychlorinated biphenyls (Svensson BG. et al., Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds, Int Arch Occup Environ Health 1994; 65(6) 351-8), infectious diseases, in particular tuberculosis (Restrepo LM. et al., Natural killer cell activity in patients with pulmonary tuberculosis and in health controls, Tubercle 1990 Jun; 71(2): 95-102),

chronic fatigue syndrome (CFS) (Whiteside TL, Friberg D, Natural killer cells and natural killer cell activity in chronic fatigue syndrome, Am J Med 1998 Sep 28; 105(3A): 27S-34S), and all parasitic infections, such as, for example, schistosomula (Feldmeier H, et al., Relationship between intensity of infection and immunomodulation in human schistosomiasis. II. NK cell activity and in vitro lymphocyte proliferation, Clin Exp Immunol 1985 May; 60(2): 234-40).

Thus, the objective is to obtain novel antibodies that are more effective compared to the current antibodies, which would make it possible to envision their use in therapy for pathologies in which there are few expressed molecular targets or a low antigenic density and also a limited number of effector cells capable of being activated.

We had shown, in our application WO 01/77181 (LFB), the importance of selecting cell lines that make it possible to produce antibodies having a strong ADCC activity via FcγRIII (CD16). We had found that modifying the glycosylation of the constant fragment of the antibodies produced in rat myeloma lines such as YB2/0 resulted in the ADCC activity being improved. The glycan structures of said antibodies are of the biantennary type, with short chains, a low degree of sialylation, nonintercalated terminal attachment point mannoses and GlcNAcs, and a low degree of fucosylation.

Now, in the context of the present invention, we have discovered that the advantage of having a strong affinity for CD16 can be further enhanced by additional conditions aimed at producing antibodies which also induce the production of cytokines, in particular the production of IFNγ or IL2, by the cells of the immune system.

The abovementioned two characteristics complement one another. Specifically, the production of IFNγ or IL2 induced by the antibodies selected by means of the method of the invention can enhance the ADCC activity. The mechanism of action of such an activation probably stems from a positive autocrine regulation of the effector cells. It may be postulated that the antibodies bind to CD16, bringing about a cytotoxic activity, but also induce the production of IFNγ or IL2 which, in the end, results in an even greater increase in the cytotoxic activity.

We show here that the optimized antibodies of the invention maintain good effectiveness even when the antigenic density or the number of effector cells is limited therapy is low. It is now possible to treat pathologies for which an antibody treatment could not be envisioned up until now.

Description

Thus, the invention relates to the use of an optimized human or humanized chimeric monoclonal antibody, characterized in that:

a) it is produced in a cell line selected for its properties of glycosylation of the Fc fragment of an antibody, or

b) the glycan structure of the Fcgamma has been modified ex vivo, and/or

c) its primary sequence has been modified so as to increase its reactivity with respect to Fc receptors;

said antibody having i) a rate of ADCC via FcγRIII (CD16) of greater than 50%, preferably greater than 100%, for an E/T (effector cell/target cell) ratio of less than 5/1, preferably less than 2/1, compared with the same antibody produced in a CHO line; and ii) a rate of production of at least one cytokine by a CD16 receptor-expressing effector cell of the immune system of greater than 50%, 100%, or preferably greater than

200%, compared with the same antibody produced in a CHO line;

for preparing a medicinal product intended for the treatment of pathologies for which the number of antigenic sites or the antigenic density is low, or the antigens are relatively inaccessible to antibodies, or else for which the number of activated or recruited effector cells is low.

Advantageously, the number of antigenic sites is less than 250 000, preferably less than 100 000 or 50 000 per target cell.

Said cytokines released by the optimized antibodies are chosen from interleukins, interferons and tissue necrosis factors (TNFs).

Thus, the antibody is selected for its ability to induce the secretion of at least one cytokine chosen from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, etc., TNFa, TGFβ, IP10 and IFNγ, by the CD16 receptor-expressing effector cells of the immune system.

Preferably, the antibody selected has the ability to induce the secretion of IFNγ or of IL2 by the CD16 receptor-expressing effector cells of the immune system, or of IL2 by Jurkat CD16 cells, for a low number of antigenic sites present at the surface of the target cells or for a low number of antigens accessible to antibodies. The amount of IFNγ or of IL2 secreted reflects the quality of the antibody bound by the CD16 receptor, as regards its antigen-binding integrity (Fc function) and effectiveness (antigenic site). In addition, the secretion of IFNγ or of IL2 by the cells of the immune system can activate the cytotoxic activity of the effector cells. Thus, the antibodies of the invention are also useful for the treatment of pathologies for which the number of activated or

recruited effector cells is low.

The effector cells can express an endogenous CD16 or can be transformed. The term "transformed cell" is intended to mean a cell that has been genetically modified so that it expresses a receptor, in particular the CD16 receptor.

In a particular embodiment, the antibody of the invention is capable of inducing the secretion of at least one cytokine by a leukocytic cell, in particular of the NK (natural killer) family, or by cells of the monocyte-macrophage group. Preferably, for selecting the antibodies, a Jurkat line transfected with an expression vector encoding the CD16 receptor is used as effector cell. This line is particularly advantageous since it is immortalized and develops indefinitely in culture media. The amount of interleukin IL2 secreted reflects the quality of the antibody bound by the CD16 receptor, as regards its antigen-binding integrity (Fc function) and effectiveness (antigenic site).

In another embodiment, the optimized antibody can be prepared after having been purified and/or modified ex vivo by modification of the glycan structure of the Fc fragment. To this effect, any chemical, chromatographic or enzymatic means that is suitable for modifying the glycan structure of antibodies can be used.

In another embodiment, the antibody can be produced by cells of rat myeloma lines, in particular YB2/0 and its derivatives. Other lines can be selected for their properties of producing the antibodies defined above. Human lymphoblastoid cells, insect cells and murine myeloma cells may, for example, be tested. The selection may also be applied to the evaluation of antibodies produced by transgenic plants or transgenic mammals. To this effect, production in CHO serves as a

reference (CHO being used for the production of medicinal product antibodies) for comparing and selecting the production systems producing the antibodies according to the invention.

The general glycan structure of antibodies corresponds to a biantennary type, with short chains, a low degree of sialylation, nonintercalated terminal attachment point mannoses and GlcNAcs, and a low degree of fucosylation. In these antibodies, the intermediate GlcNac content is non zero.

Thus, the invention is directed toward the use of an antibody described above, for preparing a medicinal product intended for the treatment of a pathology which escapes the immune response, in particular chosen from hemolytic disease of the newborn, Sézary syndrome, chronic myeloid leukemias, cancers in which the antigenic targets are weakly expressed, in particular breast cancer, pathologies associated with the environment that target in particular individuals exposed to polychlorinated biphenyls, infectious diseases, in particular tuberculosis, chronic fatigue syndrome (CFS), and parasitic infections such as, for example, schistosomula.

Example 1: ADCC induced by anti-Rhesus antibodies as a function of the number of antigenic sites

The same sequence encoding an IgG1 specific for the Rhesus D antigen is transfected into CHO and YB2/0. The cytotoxic activity of the antibodies is compared with respect to Rhesus-positive red blood cells expressing at their surface various amounts of Rhesus antigen, i.e.: normal O+ red blood cells (10-20 000 sites) and red blood cells overexpressing the Rhesus antigen (> 60 000 sites).

The results are given in figure 1:

The ADCC activity of the antibodies expressed in CHO (triangle) or YB2/0 (square) on normal red blood cells (N, open) or red blood cells overexpressing the Rhesus antigen (GR6, solid) are compared.

Example 2: ADCC induced by anti-HLA-DR antibodies as a function of the amount of effectors

The same sequence encoding an IgG1 specific for the HLA-DR antigen is transfected into CHO and YB2/0. The cytotoxic activity of the antibodies is compared with respect to the Raji cell in the presence of various effector/target ratios (see figure 2).

The difference in cytotoxic activity between the optimized antibody expressed by YB2/0 and CHO increases as the E/T ratio decreases. Thus, for the following ratios, 20/1; 10/1; 5/1; and 2/1, the relative percentage lysis induced by the antibody expressed in CHO (100% being the value of the antibody expressed in YB2/0 for each ratio) is 61%, 52%, 48% and 36%, respectively.

The antibody expressed in YB2/0 proves to be more cytotoxic than when it is produced by CHO under conditions with low amounts of effectors.

Example 3: ADCC induced by anti-HLA-DR antibodies as a function of the amount of accessible antigens

The same sequence encoding an IgG1 specific for the HLA-DR antigen is transfected into CHO and YB2/0. The cytotoxic activity of the antibodies is compared with respect to the Raji cell in the presence of various effector/target ratios (E/T ratio).

The cytotoxic activity of the antibodies is compared with respect to Raji cells for which the antigenic sites have been blocked beforehand with increasing

amounts of an inactive (non-cytotoxic) anti-HLA-DR murine antibody, so as to have a decreasing number of HLA-DR antigens available with respect to the antibodies to be evaluated (see figure 3).

The fewer available antigenic sites there are, the greater the difference in cytotoxic activity between the optimized antibody produced in YB2/0 and the antibody produced in CHO. This indicates that one of the applications of the optimized antibody may concern target cells expressing at their surface a weakly expressed antigen recognized by the therapeutic antibody. This provides a clear therapeutic advantage compared with an antibody expressed in a CHO-type cell.

Example 4: Production of IL2 by Jurkat CD16, induced by anti-HLA-DR antibodies, as a function of the amount of accessible antigens

The same sequence encoding an IgG1 specific for the HLA-DR antigen is transfected into CHO and YB2/0. The activation of the effector cell (secretion of IL2 by Jurkat CD16) induced by the antibodies is compared with respect to Raji cells for which the antigenic sites have been blocked beforehand with increasing amounts of a murine anti-HLA-DR antibody, so as to have a decreasing number of HLA-DR antigens available with respect to the antibodies to be evaluated (see figure 4).

Example 5: ADCC induced by anti-CD20 antibodies as a function of the amount of antigens

The results obtained with the anti-CD20 in ADCC confirm those obtained with the anti-HLADR, i.e. the lower the number of antigenic sites that are available and expressed at the surface of the target cells, the greater the difference in activation of the effector cells between the optimized antibody produced by YB2/0

and the antibody produced in CHO.

Example 6: Production of IL2 by Jurkat CD16, induced by anti-CD20 antibodies, as a function of the amount of accessible antigens

The same sequence encoding an IgG1 specific for the CD20 antigen is transfected into CHO and YB2/0. The activation of the effector cell (secretion of IL2 by Jurkat CD16), induced by the antibodies, is compared with respect to Raji cells for which the antigenic sites have been blocked beforehand with increasing amounts of an inactive murine anti-CD20 antibody, so as to have a decreasing number of CD20 antigens available with respect to the antibodies to be evaluated (see figure 5).

The fewer available antigenic sites there are, the greater the difference in activation of the Jurkat CD16 cells, induced by the optimized antibody produced by YB2/0 and the antibody produced in CHO. This means that a cell expressing a low antigenic density can nevertheless induce the activation of an effector cell via an optimized antibody. This capacity is much more restricted, or even zero, with an antibody expressed in CHO.

The therapeutic applications of the optimized antibody, i.e. the antibody produced in YB2/0, may thus relate to target cells expressing at their surface a weakly expressed antigen.

In conclusion, the optimized antibodies prove to be particularly useful for therapeutic applications when the target cells express few antigens at their surface, whatever the antigen.

CLAIMS

1. The use of an optimized human or humanized chimeric monoclonal antibody, characterized in that:
a) it is produced in a cell line selected for its properties of glycosylation of the Fc fragment of an antibody, or
b) the glycan structure of the Fcgamma has been modified ex vivo, and/or
c) its primary sequence has been modified so as to increase its reactivity with respect to Fc receptors;
said antibody having i) a rate of FcγRIII (CD16)-dependant ADCC of greater than 50%, preferably greater than 100%, for an E/T (effector cell/target cell) ratio of less than 5/1, preferably less than 2/1, compared with the same antibody produced in a CHO line; and ii) a rate of production of at least one cytokine by a Jurkat CD16 effector cell or by a CD16 receptor-expressing effector cell of the immune system of greater than 50%, 100%, or preferably greater than 200%, compared with the same antibody produced in a CHO line;
for preparing a medicinal product intended for the treatment of pathologies for which the number of antigenic sites or the antigenic density is low, or the antigens are relatively inaccessible to antibodies, or else for which the number of activated or recruited effector cells is low.

2. The use as claimed in claim 1, characterized in that the number of antigenic sites is less than 250 000, preferably less than 100 000 or 50 000 per target cell.

3. The use as claimed in either of claims 1 and 2, characterized in that said cytokines released by the optimized antibodies are chosen from interleukins, interferons and tissue necrosis factors (TNFs).

4. The use as claimed in either of claims 1 and 2, characterized in that the optimized antibody induces the secretion of at least one cytokine chosen from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, etc., TNFa, TGFβ, IP10 and IFNγ, by the effector cells of the immune system, in particular those expressing the CD16 receptor.

5. The use as claimed in either of claims 1 and 2, characterized in that the antibody induces the secretion of IL-2 by Jurkat CD16 cells or of IFNγ and IL2 by the CD16 receptor-expressing effector cells of the immune system, for a low number of antigenic sites present at the surface of the target cells or for a low number of antigens accessible to antibodies or for a low number of effector cells.

6. The use as claimed in one of claims 1 to 5, characterized in that the effector cell is a leukocytic cell, in particular of the NK (natural killer) family, or a cell of the monocyte-macrophage group.

7. The use as claimed in one of claims 1 to 5, characterized in that the effector cell is a Jurkat cell transfected with an expression vector encoding the CD16 receptor.

8. The use as claimed in one of claims 1 to 5, characterized in that the optimized antibody is prepared after having been purified and/or modified ex vivo by modification of the glycan structure of the Fc fragment.

9. The use as claimed in one of claims 1 to 5, characterized in that the optimized antibody is produced by cells of rat myeloma lines, in particular YB2/0 and its derivatives.

10. The use as claimed in one of claims 1 to 10, characterized in that the optimized antibody has a general glycan structure of the biantennary type, with short chains, a low degree of sialylation, non-intercalated terminal attachment point mannoses and GlcNAcs, and a low degree of fucosylation.

11. The use as claimed in claim 10, characterized in that the optimized antibody has an intermediate GlcNac content that is non zero.

12. The use of an antibody as claimed in one of claims 1 to 11, for preparing a medicinal product intended for the treatment of a pathology chosen from hemolytic disease of the newborn, Sézary syndrome, chronic myeloid leukemias, cancers in which the antigenic targets are weakly expressed, in particular breast cancer, pathologies associated with the environment that target in particular individuals exposed to polychlorinated biphenyls, infectious diseases, in particular tuberculosis, chronic fatigue syndrome (CFS), and parasitic infections such as, for example, schistosomula.

Title

Treatment of pathologies which escape the immune response, using optimized antibodies

Applicant

LABORATOIRE FRANÇAIS DU FRACTIONNEMENT ET DES BIOTECHNOLOGIES

Abstract

The present invention relates to the use of optimized human or humanized chimeric monoclonal antibodies which are produced in selected cell lines, said antibodies having strong affinity for the CD16 receptor of the effector cells of the immune system, and also being able to induce the secretion of cytokines and of interleukins, in particular IFNγ or IL2, for the treatment of pathologies for which the target cells express only a low antigenic density and in which the effector cells can only be recruited in small amounts.

ADCC on red blood cells: comparison of normal red blood cells (N) versus red blood cells overexpressing the Rhesus antigen (GR6).
(Teg 500 µg/well, ADCC 375 03 017)

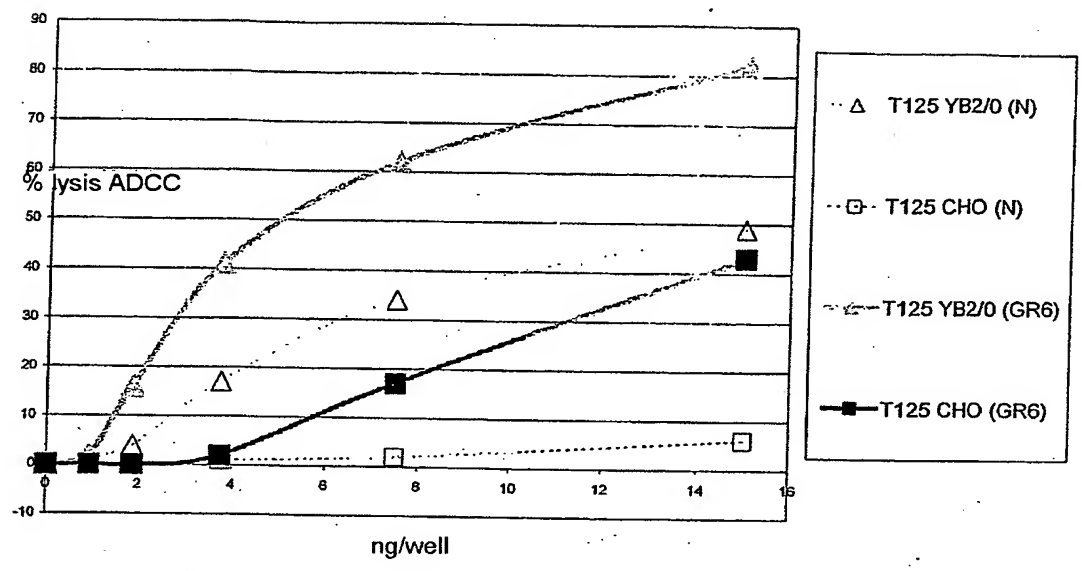

FIGURE 1

ADCC activity induced by the anti-HLA-DR chimeric antibodies expressed in CHO and YB2/0 as a function of the E/T ratio

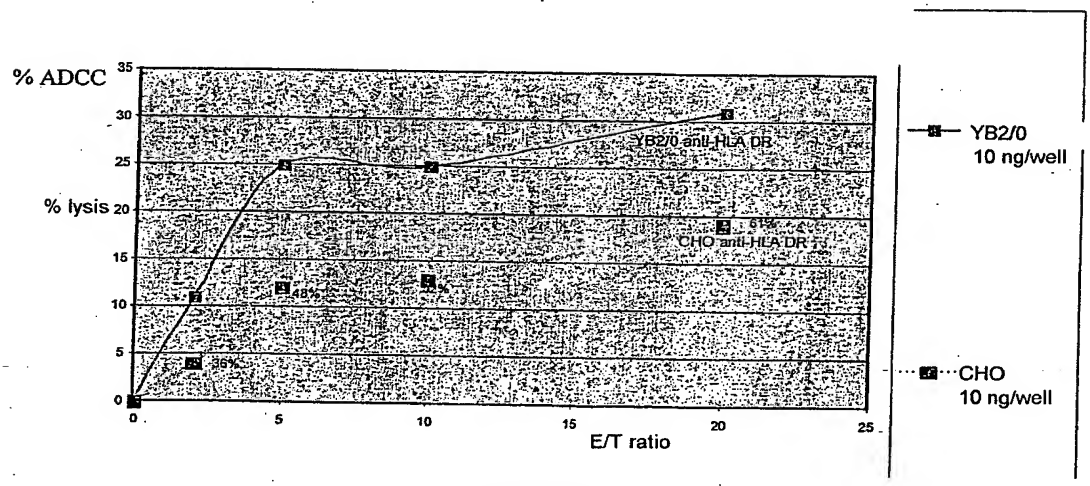

FIGURE 2

Influence of the number of HLA-DR antigens expressed on Raji (blockade with Lym-1) on the ADCC activity induced by the anti-HLA-DR chimeric antibodies expressed in CHO (square) or YB2/0 (triangle)

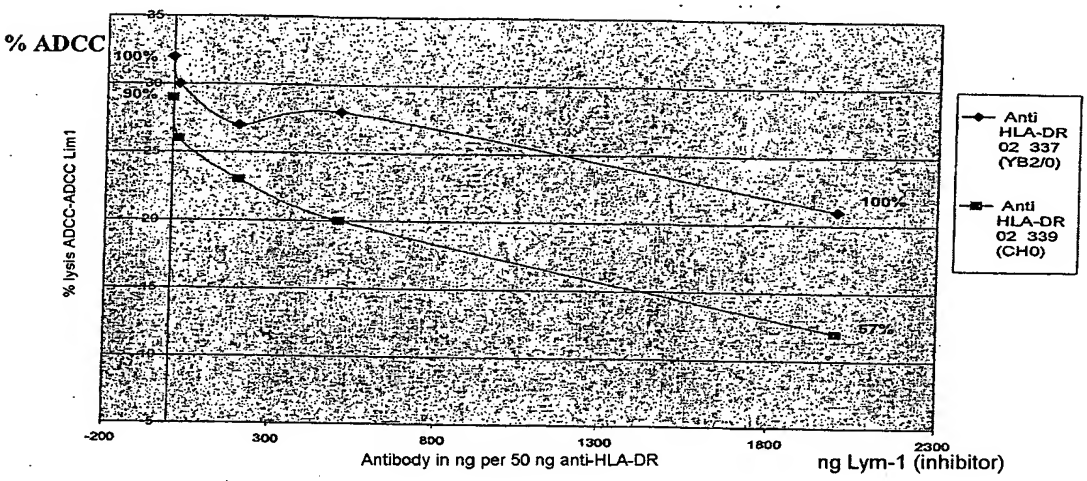

FIGURE 3

Influence of the number of HLA-DR antigens expressed on Raji (blockade with Lym-1) on the activation of Jurkat CD16 (IL2) induced by the anti-HLA-DR chimeric antibodies expressed in CHO (square) or YB2/0 (triangle)

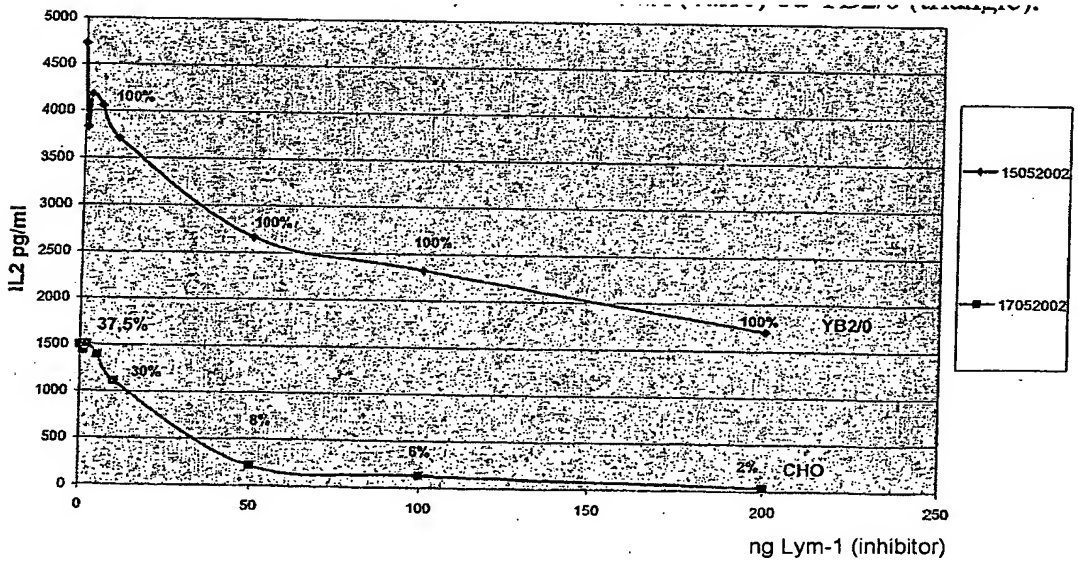

FIGURE 4

Influence of the number of CD20 antigens expressed on Raji (blockade with CAT 13) on the activation of Jurkat CD16

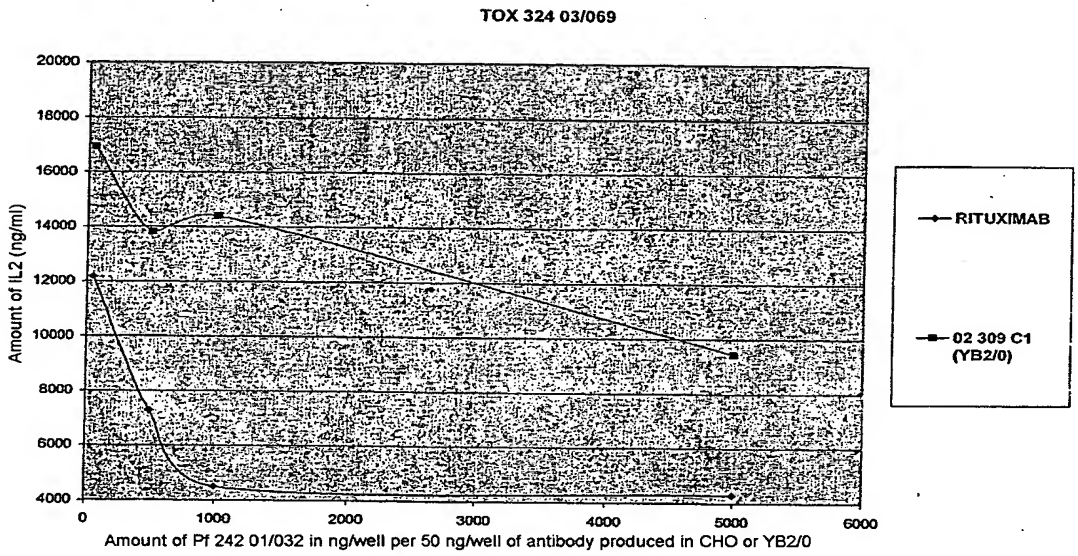

FIGURE 5